In silico studies on potential binding sites of amyloid inhibitor compounds on amyloid beta peptide

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by

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CERTIFICATE

This is to certify that the thesis entitled "***In silico* studies on potential binding sites of amyloid inhibitor compounds on amyloid beta peptide''** submitted by **Mr. Sumon Rudra** in partial fulfillment of requirement of **B.Tech** Degree in **Biotechnology Engineering** at the **NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA** is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Date:

Dr. Nandini Sarkar
(Supervisor)

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Contents

List of figures

List of Tables

ABSTRACT

The formation of structurally similar insoluble fibrillar protein aggregates, called amyloids, is known to cause several neuronal and non-neuronal degenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and Diabetes mellitus type II. In the present scenario, there is still an absence of efficient and medically approved therapeutic agent which could effectively inhibit the formation of amyloid fibrils. The similarity in structure of the amyloid fibrils of different proteins can be exploited to device a common mechanism to inhibit its formation. However, in aqueous solution their tendency to aggregate hinders the effective conformational studies. Thus in the present study we employ *in silico* approach to explore the inhibition mechanism of amyloidogenesis based on the interaction of an amyloidogenic protein with certain small molecules which have been demonstrated to effectively inhibit fibrillar assemblies. We have chosen an amyloidogenic protein called Beta-amyloid (1-42) which is found to play a crucial role in the onset of Alzheimer's disease. The small molecules chosen here are mostly polyphenol molecules which have been found to, specifically and efficiently, inhibit amyloid fibrillogenesis *in vitro* and attenuate their associated cytotoxicity. Based on the results obtained we attempt to propose a mechanism by which these small molecules inhibit the fibril formation which might be relevant in future for *de novo* synthesis of small molecule inhibitors for the treatment of amyloidosis.

INTRODUCTION

One of the most important and startling biological phenomena is the folding of a nascent protein into its native three-dimensional structure. The understanding of this complex process elucidates the way in which the properties of a molecular system are influenced by evolutionary selection for advantageous functions. The key factor behind the amazing diversity in the chemical processes of living system is the process of protein folding which leads to generation of a number varied and highly specific structures that brings closer important functional groups.

Protein folding is not only crucial for the biological activity of the protein itself but also is important for a variety of other cellular processes, which include the molecular trafficking to specific cellular destinations and cellular growth regulation and differentiation. It is the native structure of the protein that enables it survive in the complex biological environment and interact with selectively with other bio-molecules in the surrounding. It is therefore obvious that the failure of proteins to fold into their native state, or to remain in their native state, can disrupt normal biological functions and cause a very wide variety of pathological conditions [1]. In a highly crowded environment, intermolecular interactions may overcome intramolecular ones and these misfolded proteins may interact with each other to form aggregates. These aggregates are found to be amorphous or highly ordered. The highly ordered form is called "amyloid fibril". Amyloid formation varies greatly with environmental conditions like temperature, pH, oxidative stress, ionic strength, peptide concentration etc. These highly ordered protein fibrils form aggregates and deposition of which leads to several degenerative diseases such as Alzheimer's disease, Parkinson's disease, Type II diabetes and so on. Thus it is necessary to identify compounds that can potentially inhibit or prevent aggregation and amyloid formation effectively. The understanding of their mechanism of action can serve as a key therapeutic approach towards amyloid diseases.

In the present study, we have employed in silico approach to identify potential binding sites of selected amyloid inhibitor compounds towards amyloidogenesis of amyloid beta (Aβ) peptide, a

key peptide which is involved in amyloid formation in brain tissues leading to Alzheimer's disease. We have further tried to elucidate the mechanism of action of these inhibitor compounds towards Aβ amyloidogenesis. These studies will throw some light towards key interactions which play a vital role in blocking protein aggregation and preventing amyloid formation and thus can provide essential information towards development of effective therapeutics against amyloidosis.

LITERATURE REVIEW

Protein Folding and Misfolding in a cell

The translation of proteins from the respective genes occurs in the Ribosomes. It is after the synthesis of the protein that the folding process starts. However, in some cases, folding *in vivo* may be initiated even before the translation process is over, when the nascent polypeptide chain is still not yet released from the ribosome. As earlier discussed the protein folding process is very sensitive to the cellular environment. Misfolded proteins might have a tendency to interaction with other molecules within the crowded environment of a cell [1]. This is because of the fact that core regions buried in the native state get exposed to the solvent. Since the living system are the most complicated and yet efficient machinery, there ought to be a proof reading mechanism for the misfolding of the proteins. This role is played by the molecular chaperones that are present in all cells and cellular compartments. They play a crucial role in the folding of the proteins by interacting with nascent chains as they are being released from the ribosome, and by guiding future stages of the folding.

In eukaryotic systems, many proteins that are synthesized in a cell are released into the extracellular environment, while some of them are translocated into the Endoplasmic Reticulum(ER), where they fold correctly into their native state before releasing through the Golgi apparatus. There are various types of chaperones and folding catalysts present in the ER, which facilitate ‘quality-control’ check on the proteins before they are exported [2]. The properly folded proteins are directed to the Golgi complex and then to the extracellular environment, while, misfolded proteins are transported in a different pathway (the unfolded protein response) in which they may be correctly folded or degraded in the cytoplasm by proteasomes.

As it is known clearly that most important function such as translocation across membranes, immune response, cell cycle regulation etc., are performed by proteins which owe their functionality to their native three-dimensional folded structure that facilitates their binding to various molecules. Thus, it is imperative that misfolding or unfolding of the proteins must be

ensured quality for maintaining normal function of the cell. However, as we know, no system is perfect. There might be a slip even in this intricate machinery which may lead to failure of proper of folding of proteins and cause several diseases [3,4]. Such diseases can be broadly classified into two categories.

In first category, protein misfolding and degradation by proof reading machinery of the cell results in loss of the function of the protein or the protein itself. For example, in cystic fibrosis, ΔF508 mutation occurs in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein. This causes protein misfolding and subsequently it is degraded by ubiquitin-proteasome pathway [5]. The α1-antitrypsin (α 1-AT) deficiency is also exemplary in the same class [6]. It is noteworthy that the epithelial cells of many organs like lungs, liver, digestive tract, etc. comprises the CFTR protein which facilitates the transport of chloride ions in epithelial cells to the outside layer of mucus. Moreover, α1-antitrypsin produced in the liver, is found to inhibit the action of neutrophil elastase enzyme, which can disrupt connective tissue and damage the lungs.

The second category comprises amyloidongenic diseases, i.e., disease which are caused due to aggregation or amyloid formation facilitated by misfolding of the protein [7,8]. The Insoluble protein aggregates or amyloids are toxic to the cell and it has been reported that the deposition in important organs and tissue is directly influential in the causation of a number of disorders, including Alzheimer's and Parkinson's diseases, the spongiform encephalopathies and type II diabetes [9]. In the next section we look at the formation of these species.

Protein aggregation and amyloid formation

The wide variety of diseases associated with protein misfolding owe their generation to the conversion of soluble functional peptides or proteins into highly organized insoluble fibrillar aggregates. These structures are generally described as amyloid fibrils or plaques when they accumulate extracellularly, whereas, when fibrils are formed inside the cell ,the term "intracellular inclusions" is commonly used [7].Amyloidosis is caused by the abnormal deposition of amyloid in organs which may be a cause of various neurodegenerative diseases. The amount of deposition of

protein aggregates in organs may greatly vary from undetectable miniscule quantities (in neurodegenerative diseases), to even kilograms of protein.

Although it is clear that amyloidosis is a result of amyloid deposition in the tissues, but there still is a need to determine the specific molecules and precise mechanisms of the pathogenesis and the mode of action. One of the many suggested pathways is the disruption of tissue architecture which comprises a direct interaction of the insoluble fibrillar deposits with the cell membrane. This leads to the creation of pores which result in the permeabilization of the membrane and eventually cell death [10]. Other hypotheses emphasize on the toxicity of amyloid aggregates. Some studies indicate variation of the intracellular ion content and changes in redox status maybe caused by the deposition of toxic aggregates in the cell. This could be stated as a consequence which the cell experiences when it is exposed to membrane permeability caused by purification [11]. Whereas in some cases, normal organic functions are supposedly disrupted due to direct deposition of heavy masses (few Kgs) of fibrils .

It has been found that amyloid fibrils are formed by a wide variety of proteins which negates the earlier assumption of involvement of only few specific proteins associated with known clinical disorders. *In vitro* synthesis of amyloid fibrils by using molecules as myoglobin, and also by homopolymers such as polythreonine or polylysine provides a compelling evidence for the above statement [12]. The charge of the molecule, secondary-structure and number of hydrophobic surfaces influence the relative aggregation rates for a wide range of peptides and proteins [13]. The proper folding of a globular protein ensures the burial of the hydrophobic side chains and the main polypeptide chain into a core region .Denaturing conditions like low pH, high temperature etc., cause the unfolding of these proteins and leads to the exposure of the hydrophobic core to the solvent. These unfolded or misfolded proteins may be degraded by proteolysis or will interact to form amyloid fibrils .A nascent polypeptide chain, after its release from the ribosome, may either fold into its native 3D structure,(by sequentially folding into different Intermediates), or it may misfold and face consequences such as degradation or aggregation. An amyloid fibril has a unique well-ordered misfolded structure. X-ray diffraction studies of other *in vitro* assemblies indicate the presence of natively folded molecules akin to proteins, in oligomers that are functional, macro-

molecular complexes and naturally occurring protein fibres. The relative thermodynamic and kinetic stability governs the interconversion of the various states under any given conditions. In living systems, however, the cellular environment, molecular chaperones and proteolytic enzymes play a key role in influencing the transitions between the different states. It is, thus, evident that failure of these regulatory mechanisms may cause misfolding diseases [14].

Research work over many years has identified different proteins which cause formation of amyloid fibrils in the tissues which leads rise to several diseases in human. A list of some of the amyloidogenic proteins and the diseases caused by them is presented in Table1 [15] below:

Disease	**Protein featured**	**Official abbreviation**
Alzheimer's disease	Beta amyloid	Aβ
Diabetes mellitus type 2	IAPP (Amylin)	AIAPP
Parkinson's disease	Alpha-synuclein	none
Transmissible spongiform encephalopathy	PrP	APrP
Huntington's Disease	Huntingtin	none
Medullary carcinoma of the thyroid	Calcitonin	ACal
Cardiac arrhythmias, Isolated atrial amyloidosis	Atrial natriuretic factor	AANF
Atherosclerosis	Apolipoprotein AI	AApoA1
Rheumatoid arthritis	Serum amyloid A	AA
Prolactinomas	Prolactin	APro
Cerebral amyloid angiopathy	Beta amyloid	Aβ

The structurally similar amyloids are formed by a structurally diverse group of proteins. This observation gives rise to the speculation that can there be a common mode of therapy against all amyloid diseases?

Amyloid biophysics

Amyloid exhibits a cross-beta sheet quaternary structure. A developed amyloid fibril comprises 2-5 protofibrils which form helical rope like structure by twisting around each other. The diameter of this structure varies from 7-30 nm. The core of amyloid protofilament constitutes the cross β sheet structure. Cross β sheet interaction stabilizes each protofilament (comprising beta-strands) which are perpendicular to the fibrillar axis [15]. The ‘‘gold standard’’ test which determines the presence of the amyloid fibrils is the change in fluorescence intensity of specific dyes such as Thioflavin T (ThT) and Congo red (CR) .These dyes intercalate into the cross beta sheet structure of the fibril and thus allow them to be used as analytical tools for detection of fibrils. The breadth of the amyloid fibril is traversed by short beta-sheet ‘’stacks’’ while the length comprises aligned strands. The atomistic details of amyloid core have been revealed by recent X-ray diffraction studies performed on microcrystals. The "cross-beta" structure was thus experimentally validated with the above results which depicted short amyloidogenic regions running perpendicular to the filament axis [16].

Aβ amyloidogenesis

Alzheimer's disease, also known as Alzheimer disease (AD) in medical literature is the most common form of dementia. An uncurable disease, it is characterized by progressive neuro-dengeneration which eventually leads to death. German psychiatrist and neuropathologistAlois Alzheimer decribed it first in 1906 and the disease was thus was named after him.

In Alzheimer’s disease, the degeneration and death of the brain cells occur that process, store and retrieve information [17]. Scientific research has revealed some of the brain changes that take place in AD. Abnormal structures called beta amyloid plaques and neurofibrillary tangles are

classic biological hallmarks of the disease. Plaques form when specific proteins in the neuron's cell membrane are processed differently. Normally a transmembrane protein called Amyloid precursor protein(APP) is cleaved by an enzyme called α secretase and releases a fragment. Another enzyme γ secretase then snips another part of the APP and releases the fragment. These fragments are thought to benefit the neurons. In AD, the first cut is made by β secretase, that combined with the cut by γ secretase results in the release of short sticky fragments (30-40 amino acids) of APP called Beta-amyloid. These fragments clump together and become toxic and interfere with the function of neurons. As more fragments are added, these oligomers in increase in size and become insoluble resulting in the formation of beta-amyloid plaques. This causes disruption of cell-to-cell communication, activation of immune cells that induce inflammation and scavenging disabled cells, and, ultimately, killing cells [18].

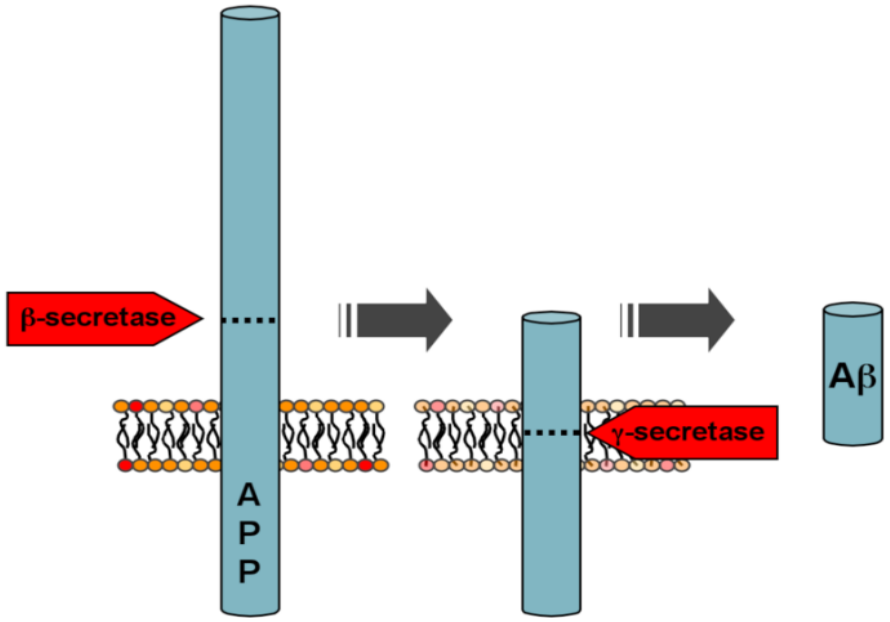


Figure 1: Mechanism of formation of amyloid beta peptide from amyloid precurcor protein. (Source:http://en.wikipedia.org/)

Beta-amyloid has generally two common isoforms: Aβ40 and Aβ4. Aβ40 is generated by cleavage that occurs in the endoplasmic reticulum, while cleavage in the trans-Golgi network generates Aβ42. It has been found that of the two, Aβ42 is the more fibrillogenici.e more likely to cause amyloid fibril formation, and induce disease states. Mutations in APP induces the increase in the relative production of Aβ42 and it has been associated with early onset Alzheimer's. Thus

scientists proposed a mechanism of Alzheimer's therapy involving modulation of the activity of β and γ secretases for the production of mainly Aβ40.

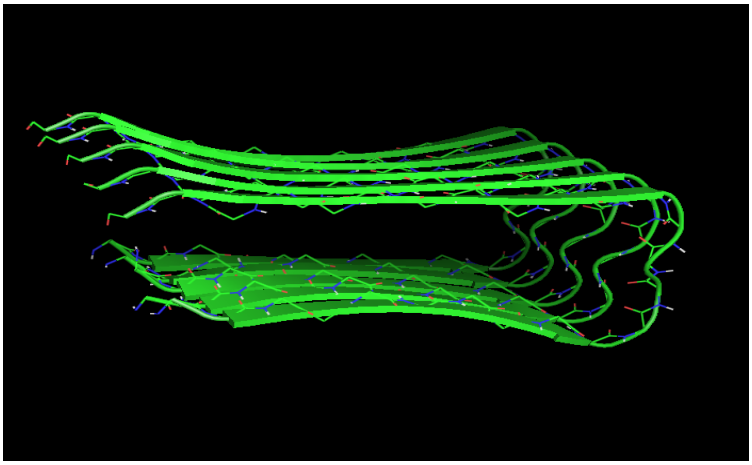


Figure 2: 3D structure of Aβ (1-42) fibrils obtained using Pymol

Therapeutic approach

Many therapeutic approaches targeting the reduction of amyloid fibrillogenesis include: inhibition of secretase activity for decreasing the production of Aβ amyloid, increasing amyloid degradation using amyloid vaccines, or preventing amyloid aggregation employing Antibodies, small peptides, or small organic molecules that may inhibit aggregation by specifically binding to the protein. Further, few small polyphenolic compounds have been reported to exhibit inhibitory effects *in vitro* and attenuation in associated toxicity of the amyloid fibrils. The early findings on the inhibitory effect of polyphenols on amyloid formation came from specific interactions of small polyphenolic compounds like Congo Red (CR) and Thioflavin T (ThT), with amyloids and inhibit its formation to some extent [19]. Later on several other aromatic phenolic compounds were found to exhibit inhibtory effect on amyloid formation *in vitro*, such as reveratrol, curcumin, rosmarinic acid, catechin, tannic acid and so on. These compounds were found to exhibit structural similarities like presence of atleast two phenolic rings and more than one –OH groups on the aromatic rings, suggesting some common mode of interaction with amyloid fibrils. This indicates involvement of some hydrophobic or ring stacking interaction of these phenolic compounds with characteristic cross-β sheet conformation present in amyloid preventing their further elongation. Also, few polyphenolic compounds have exhibited protective effects against amyloid toxicity towards cell

culture and primary culture systems, which was mainly attributed to the anti-oxidative properties of the polyphenols [20].

In vitro inhibition of amyloid formation has also been exhibited by few Quinone derivatives. Alpha-tocopherolquinone was found to inhibit amyloid beta (Aβ) fibril formation in a dose dependent manner and alleviate its toxicity in neuroblastoma cell lines. Further, pyrroloquinoline showed significant inhibitory effect on Aβ (1-42) fibrillogenesis and mouse prion protein. Several small molecules derived from Quinone were also found to be inhibit amyloidogenesis, for e.g., 1,8-dihydroxyanthraquinone was found to reduce fibril formation and neurotoxicity of β-amyloid proteins [21]. A flavonoid bicalein is found to facilitate inhibition of fibrillation of alpha-synuclein and cause disaggregation of existing fibrils [22]. Catechol derivatives such as catecholamines like dopamines and L-dopa exhibited inhibitory effect on fibrillogenesis of Aβ and α-synuclein [23,24]. Further, rifampicin and its derivatives demonstrated the effect of inhibiting toxicity of aggregates of human islet amyloid polypeptide, amylin on PC 12 cells. This was facilitated by blocking the binding sites on the cell surface which prevented the adhesion of the amylin fibrils with the cell surface [25]. For the current study eight small molecules were selected, based on literature review, which have demonstrated significant inhibitory effects on Aβ42 amyloidogenesis *in vitro*, but their mechanism of action is still unknown. These compounds are as follows:-

Curcumin: It was proposed that the reason for specific binding to the amyloid beta-peptide and facilitating the inhibition of fibril formation was the symmetric and compact structure of curcumin.[26] Its structural similarity to a beta-sheet breaker, N,N'-bis(3-hydroxyphenyl)pyridazine-3,6-diamine, named RS-0406 could be one of the reasons for its inhibition characteristics. The important structural feature of the molecule is the two aromatic end groups and any alterations in these groups was found to affect its activity. Curcumin, like chrysamine G, being lipophilic, is able to cross the blood brain barrier which facilitates its binding to plaques Its specific binding to the beta-sheet structures of the plaques formed by aggregation of various different proteins indicate that its binding is not dependent on amino acid sequence of the proteins but rather its conformation-dependent.

Resveratrol: According to certain studies, resveratrol has been found to exhibit property of free radical scavenging in a number of cellular types [27]. Free radical scavengers reduce the oxidative stress in cell environment which helps in correct folding of the proteins. It was suggested that resveratrol acts not only by its anti-oxidant ability, but also by facilitating the inhibition of Aβ fibril formation by forming hydrophobic interactions with specific residues in the polypeptide.

Benzofuran derivatives: A number of benzofuran derivatives had been identified which prevent the fibril formation in β-amyloid peptide. The inhibition mechanism comprises their binding to specific sites on the protein as determined by various assay techniques [28]. The basic ethanolamine side chain in these compounds which might be attached to the 2^{nd} or 3^{rd} position in the benzofuran ring are supposedly involved in the inhibitory action. This was deduced on the basis that derivative compounds lacking this moiety were found to lack the inhibitory action.

Hydroxyindole Derivatives: In a study, 29 indole derivatives were screened to study their inhibitory action towards fibril formation and cytotoxicity for cultured pheochromocytoma cells which is a benign tumor of sympathetic nervous system. It was found that of the 29 derivatives selected, Indole-3-carbinol, 3-hydroxyindole and 4-hydorxyindole were the most effective. The studies revealed that it was the presence of –OH group in specific position in the benzopyrrole ring interacts with the protein backbone and the different charge and electron density on the ring inhibits the π stacking interactions between the Aβ peptides, thereby blocking the elongation of the fibrils [29].

Myo-inositol: Out of the 8 naturally occurring stereoisomers, 4 are reported to be physiologically active. Myo-inositol was found to be the most abundant isomer in the brain and more recently experiments have revealed its ability to stabilize and attenuate the neurotoxic effects of Aβ42 by forming small stable micelles[30].

2,4 Dinitrophenol: The destabilization of Transthyretin(TTR) molecules results in amyloid fibril formation, which have been observed in familial amyloidotic polyneuropathy and cardiomyopathy patients.According to a research 2,4-dinitrophenol was found to exhibit highly stabilizing effect on

wild-type TTR. Similar interactions were identified with highly amyloidogenic TTR variants: TTR L55P and TTR Y78F. The stabilizing effect of the molecule was attributed to its binding capability at two hormone-binding sites of the TTR tetramer. This draws the two tetramer closer to each other thereby increasing protein stability[31].

WORK PLAN AND METHODOLOGY

Work plan

The current work comprises the following specific objectives:

- Selection of compounds exhibiting inhibitory effect on Aβ peptide amyloidogenesis *invitro* as well as *invivo.*
- Identification of amyloidogenic region(s) in the Aβ peptide using different softwares.
- Identification of binding sites between the Aβ peptide and the inhibitor compounds through molecular docking studies using docking tool.
- Identification of intermolecular interactions between Aβ peptide molecules through molecular docking.
- Comparison and analysis of the results obtained to identify key interactions between the Aβ peptideand the inhibitor molecules, and to proposea possible mechanism of inhibition of amyloidogenesis.

Methodology

Selection of amyloidogenic proteins and inhibitor compounds

Based on earlier studiesone of the amyloidogenic proteins, beta-amyloid(1-42), was selected. The pdb file (pdb id: 1IYT) of the protein was retrieved from Protein Data Bank. Similarly, few small polyphenolic compounds, which were found to exhibit amyloid inhibitory effect, were selected and

the 3D structure files of those compounds were retrieved from PubChem. The detailed list of the molecules is mentioned below in Table 2.

Identification of the amyloidogenic regions in the proteins

Amyloidogenic region of the protein was predicted using web server FoldAmyloid, which predicts the specific sequence(s) on a polypeptide chain which are likely to form aggregates based on the physicochemical properties of the amino acid residues [32].

Identification of potential binding sites between the amyloidogenic proteins and inhibitor compounds

Binding sites of the amyloidogenic proteinswere predicted by performing molecular docking using Hex software.Tools like 'Chimera' and 'PyMOL' were used to visualize the docking complex and to generate the 3D structures of the same. LigandScout software was then used to analyze the docking results.It automatically generated the images of protein-ligand interactions, depicting the various interactions of the ligand with the specific residues in the receptor.

Comparison of the results obtained by docking to explore the possibility of promiscuous inhibitor

The results obtained from docking studies were compared and analyzed to explore the possibility of finding a mechanism which might be relevant for future *de novo* inhibitors' design.

RESULTS AND DISCUSSION

Amyloid beta peptide (1-42) is a key protein involved in the onset of Alzheimer's disease. Despite extensive research on Aβ amyloidogenesis, currently no specific treatment is available against amyloidosis involving clinically approved drugs that inhibit the formation or development of existing amyloid deposit. In the current study we had attempted to understand potential binding sites between known amyloid inhibitor molecules with Aβ peptide and also to unravel the mechanism of inhibition of amyloidogenesis, through *insilico* approach.

The 3D structure of the Aβ peptide was retrieved from protein data bank. Aβ is a short peptide with 42 residues. It comprises two alpha helical regions that constitutes of residues 8-25 and 28-38 which are joined by a regular type I beta turn [33]. Further, the analysis of various proteins which were found to form amyloid fibrils revealed that there were small stretches of sequences in the proteins (amyloidogenic regions) that were mainly responsible for the amyloid formation [34]. Based on this and other studies a FoldAmyloid server was developed for prediction of amyloidogenic regions in a protein or peptide [35]. This tool to detect amyloidogenic portions in a protein had been tested on 144 amyloidogenic and 263 non-amyloidogenic peptides with good accuracy. FoldAmyloid web server was used to predict potential regions involved in amyloid formation of Aβ peptide. Two amino acid stretches from 16-21 (KLVFFA) and 32-36 (IGLMV) were found to be amyloidgenic as shown in Fig. 3.

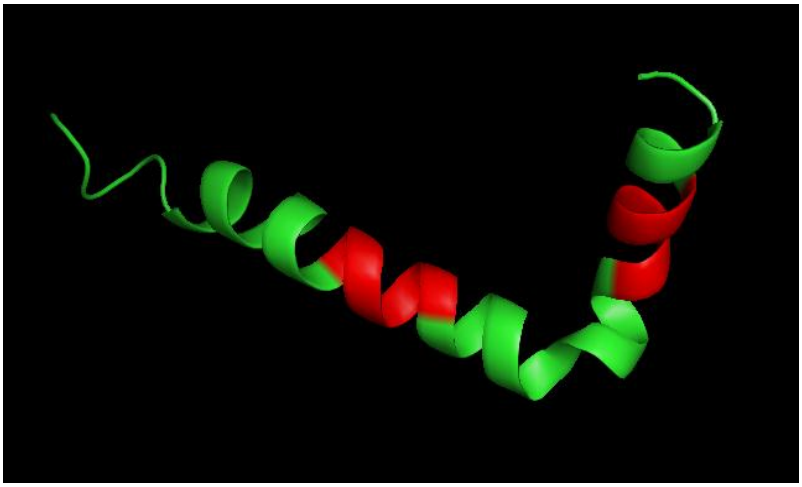


Figure 3: Solution structure of the amyloid beta-peptide 1-42 (PDB ID- 1IYT).The amyloidogenicresidues as predicted by the FoldAmyloid server (amino acid stretches 16-21 and 32-36) are highlighted in red color.

Around eight small molecule inhibitor compounds were selected based on their reported inhibitory effect on Aβ amyloidogenesis either invitro or invivo. These compounds are- 2,4dinitrophenol, 3-dihydroxyindole, 4-dihroxyindole, benzofuran, indole-3-carbinol, resveratrol, myoinositol and curcumin. All the compounds are aromatic containing one or more benzene rings. The 3D structures of eight inhibitor molecules generated using Chimera software are shown in Fig. 4.

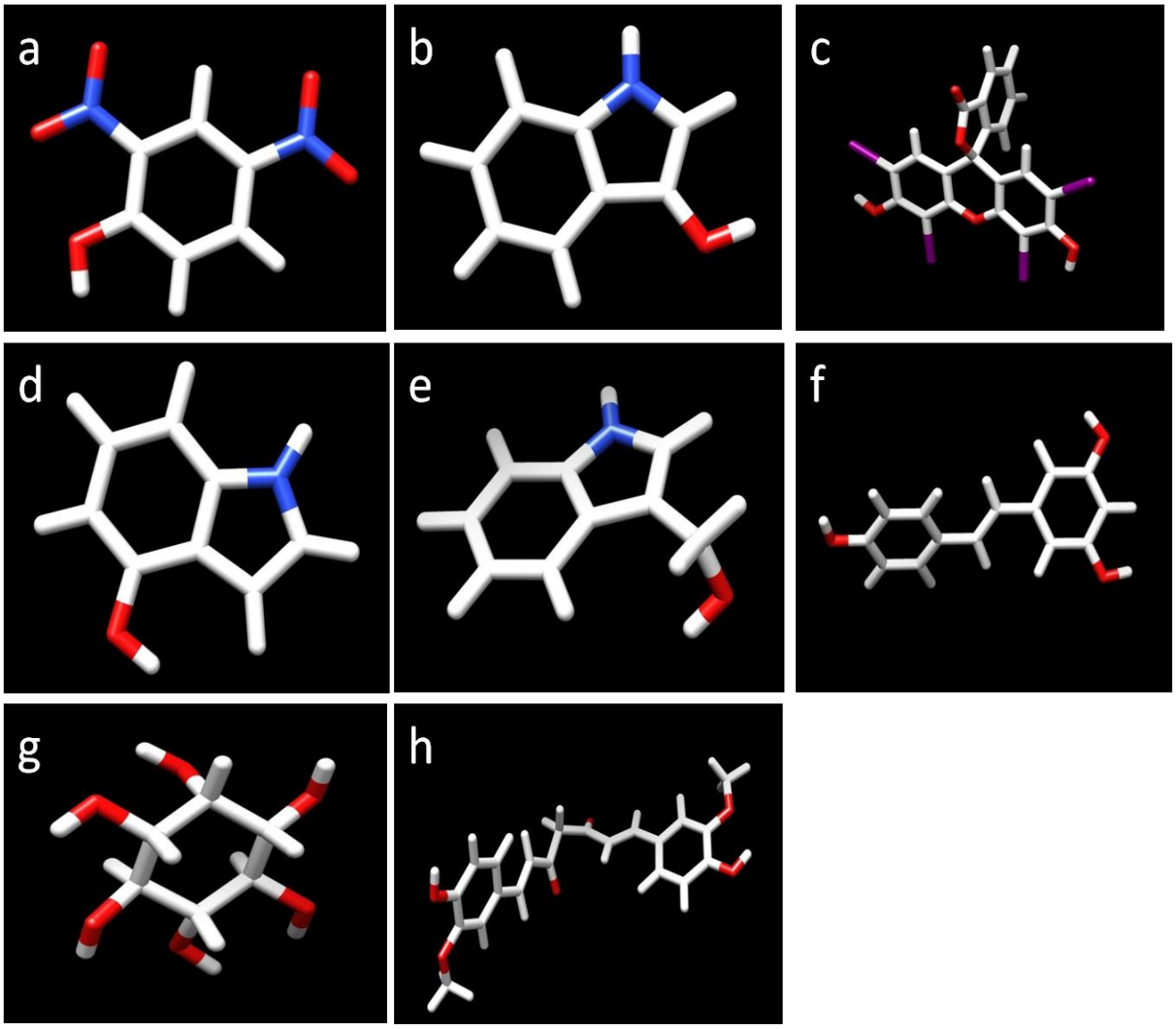


Figure 4: The 3D structures of the inhibitor molecules retrieved from PubChem and figures generated using Chimera. Following are the PubChem ID of the molecules:-**a)** 2,4 Dinitrophenol (CID 1493) **(b)**3-hydroxyindole (CID 50591) **(c)**Benzofuran derivative (CID 3259)**d)** 4-hydroxyindole (CID 75421) **(e)** Indole-3-carbinol (CID 3712) **(f)** Resveratrol (CID 445154) **(g)**Myo-inositol (CID 892) **(h)** Curcumin (CID 969516)

To identify the binding sites between Aβ peptide and the compounds molecular docking was done using Hex software. Figure 5(a) shows the docking result between Aβ peptide and 2,4dinitrophenol. The ligand binds in the vicinity of the amyloidogenic stretch KLVFFA formed by 16-21 residues. Further,Ligand Scout software was used to identify the specific interaction between Aβ peptide and 2,4 dinitrophenol, which showed specific hydrogen bonding interaction between the nitro group of the ligand and the side chain of lysine residue at position 16 of the Aβpeptide. This shows that the ligand directly interacts with the 16-21 amyloidogenic stretch and prevents aggregetaion by masking the amyloidogenic region.

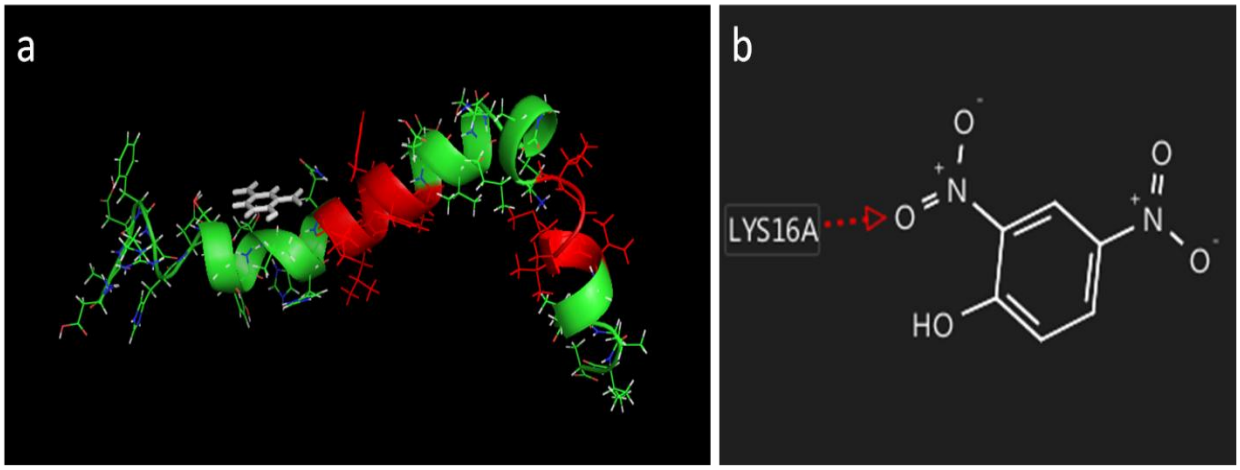


Figure 5: (a) Docking complex of Aβ peptide with 2,4dinitrophenol generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 6(a) shows the docking result between Aβ peptide and 3-hydroxyindole. Here the ligand was found to interact with the 32-36 amyloidogenic region composed of IGLMV. Ligand scout shows hydrophobic interaction between the Leu34 residue of Aβ and the indole ring of 3-hydroxyindole. Further, the blue circle over indole indicates interaction between the electron cloud of the indole ring and Met35 residue.

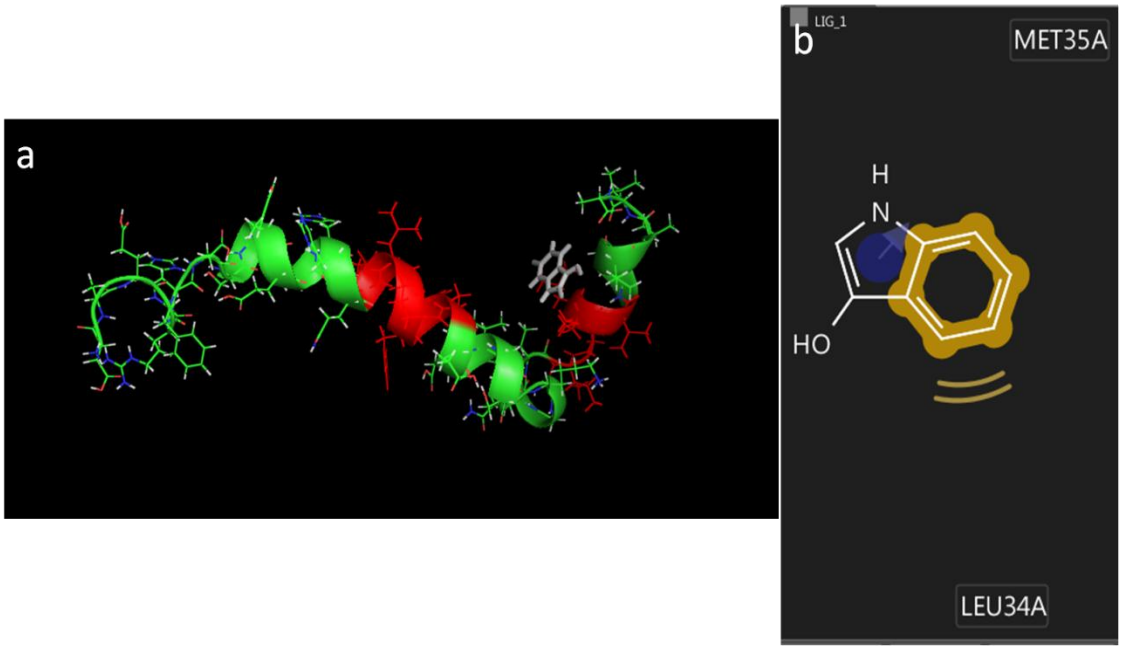


Figure 6: (a) Docking complex of Aβ peptide with 3-hydroxyindole generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 7(a) shows the docking result between Aβ peptide and 4-hydroxyindole. Here also the molecule was found to bind in the vicinity of the amyloidogenic 16-21 stretch and exhibited hydrogen bonding interaction between the hydroxyl group of the ligand and the Gln15 residue. Further, it also showed interaction between the electron cloud of the indole group and Glu11 residue.

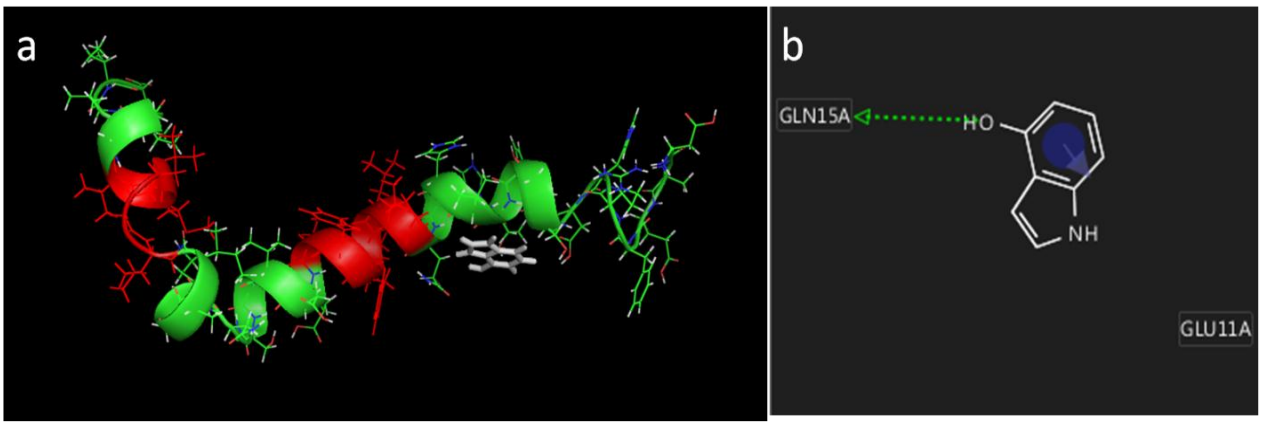


Figure 7: (a) Docking complex of Aβ peptide with 4-hydroxyindole generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 8(a) shows the docking result between Aβ peptide and a benzofuran derivative. The molecule was found to interact with both the amyloidogenic stretches of Aβ peptide. It formed specific hydrogen bonding interaction between its phenolic group and Met35 residue present in IGLMV amyloidogenic stretch. Further it formed hydrophobic interactions with the neighbouring hydrophobic amino acid residues as shown in Fig. 8(b).

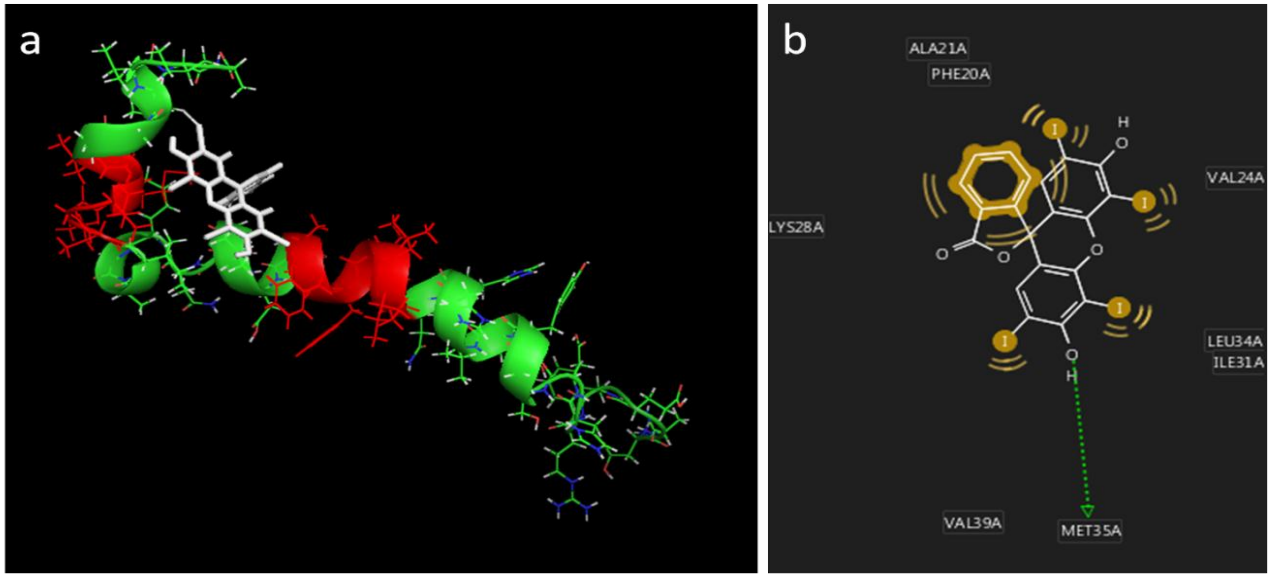


Figure 8: (a) Docking complex of Aβ peptide with benzofuran generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 9(a) shows the docking result between Aβ peptide and a curcumin. Curcumin also exhibited simultaneous interactions with both the amyloidogenic stretches of Aβ peptide. Ligand scout shows hydrophobic interactions of curcumin with five hydrophobic amino acid residues of Aβ peptide such as Met35, Val39, Phe20, Ile31 and Val24.

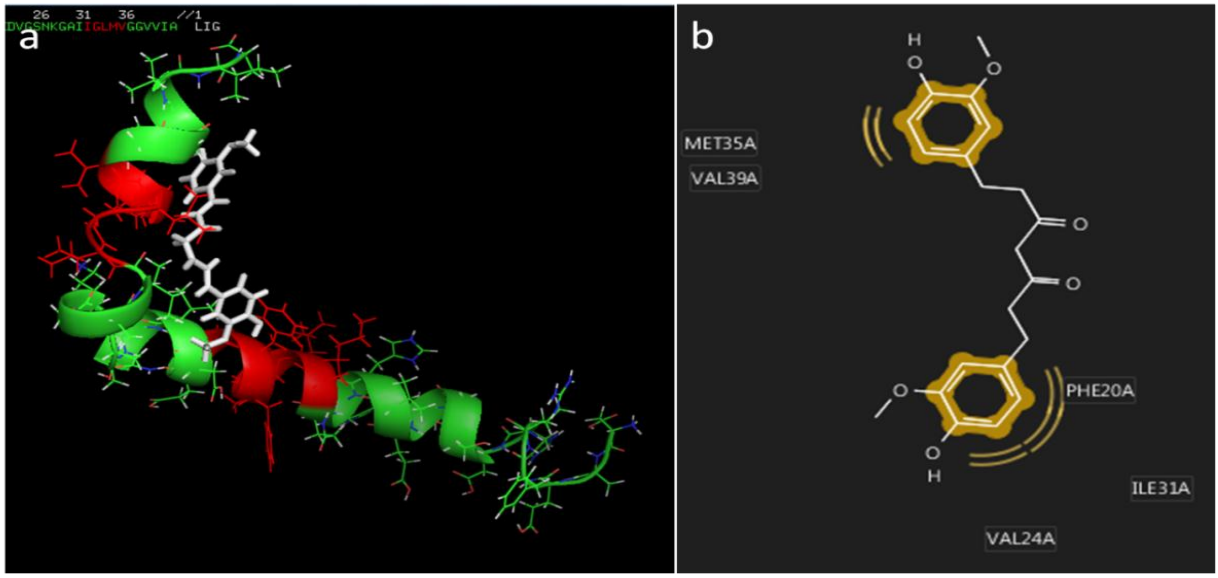


Figure 9: (a) Docking complex of Aβ peptide with curcumin generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 10(a) shows the docking result between Aβ peptide and indole-3-carbinol. Unlike other inhibitor molecules, this compound exbihited binding near the N terminal domain of the peptide far from either of the amyloidogenic stretches. Ligand scout showed hydrogen bonding interaction between the ligand and Phe4 residue. Further, electrostatic interaction was also found between the electron cloud of the benzene group of the ligand and the Arg5 residue of Aβ.

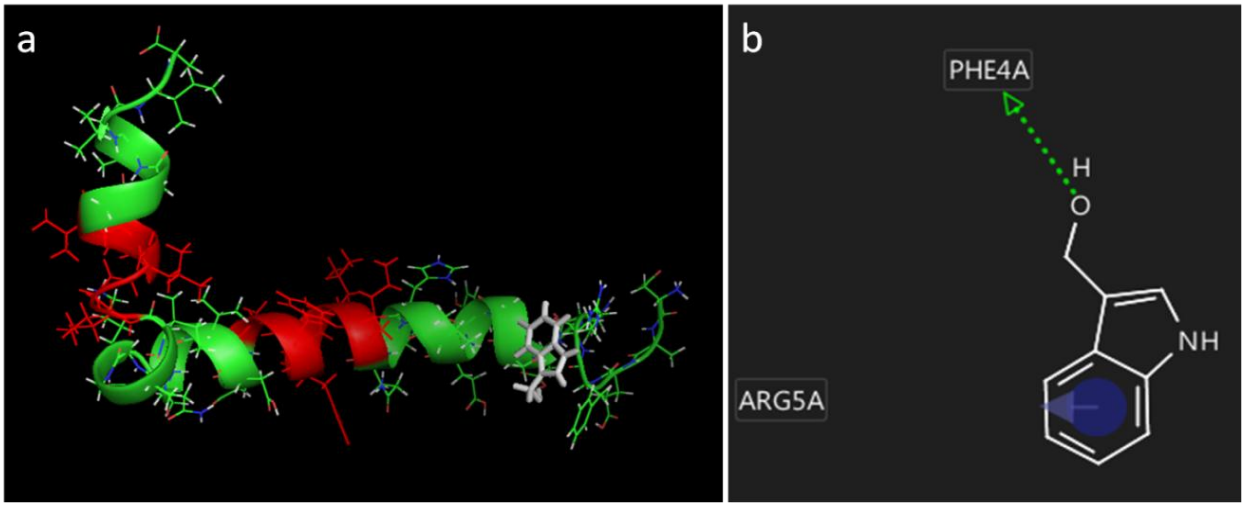


Figure 10: (a) Docking complex of Aβ peptide with indole-3-carbinol generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 11(a) shows the docking result between Aβ peptide and myoinositol. Myoinositol was found to bind with the 16-21 amyloidogenic stretch of Aβ peptide and exhibited specific hydrogen bonding interaction with Leu17 residue of the peptide as depicted by Ligand scout.

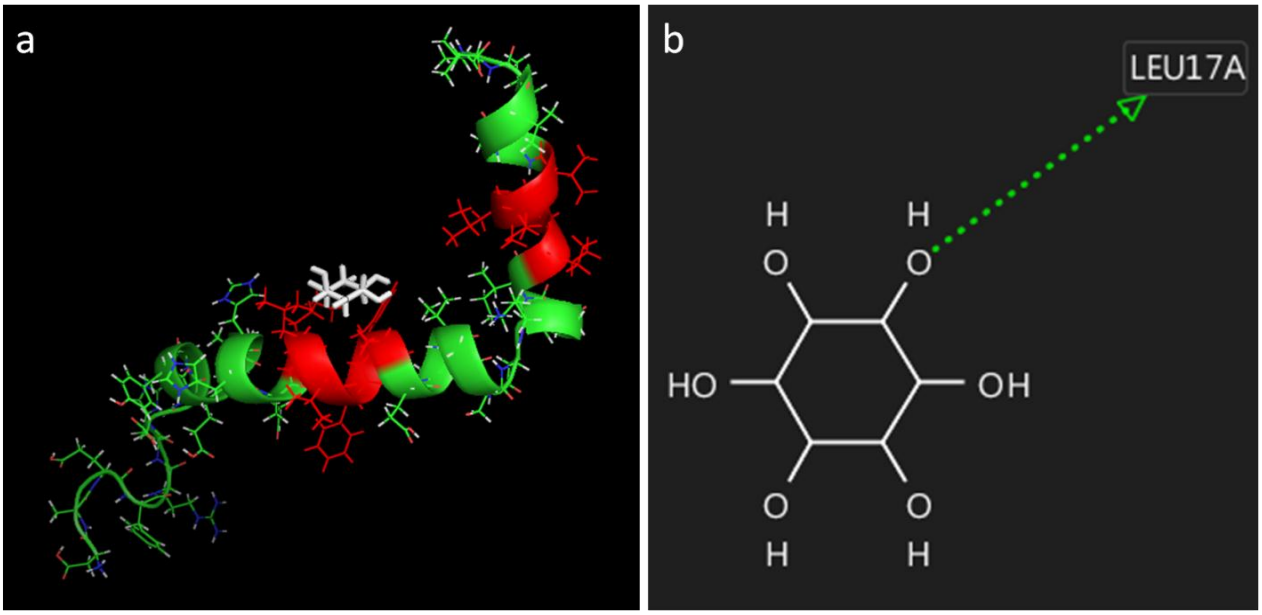


Figure 11: (a) Docking complex of Aβ peptide with myoinositol generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Figure 12(a) shows the docking result between Aβ peptide and resveratrol. This compound was found to bind near the 32-36 amyloidogenic stretch of the peptide and formed specific hydrogen bonding interaction with Met35 residue and hydrophobic interaction with Val39 residue as shown in Figure 12(b).

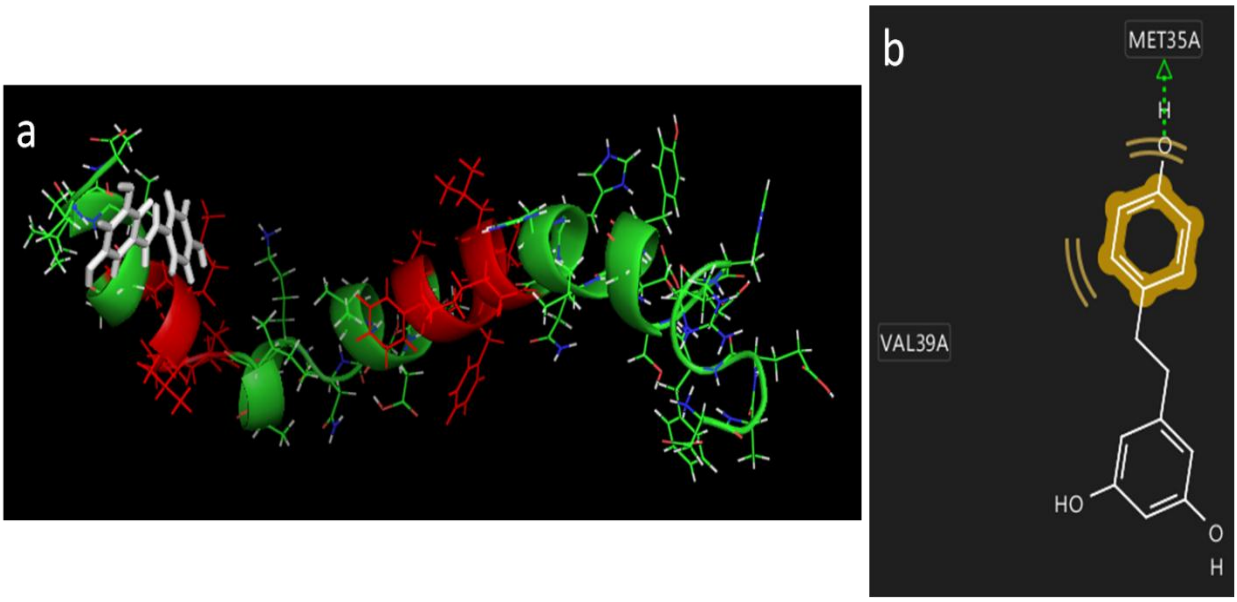
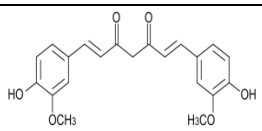
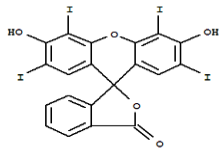
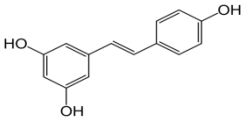
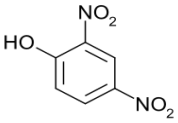
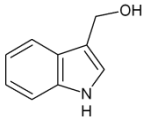
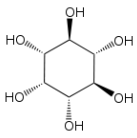
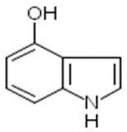
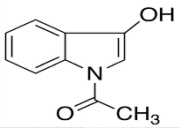


Figure 12: (a) Docking complex of Aβ peptide with resveratrol generated using Hex software (b) corresponding Ligand Scout image of the complex showing interactions with specific residues. Amyloidogenic regions in Aβ peptide as predicted by FoldAmyloid server are marked in red and the ligand is shown in white.

Thus the docking results showed that except indole-3-carbinol all the seven inhibitor molecules bound within or in the vicinity of the amyloidogenic stretches of Aβ peptide as predicted by FoldAmyloid software. This finding suggests a possible amyloid inhibitory mechanism of these compounds by binding and masking the amyloidogenic region(s) in the peptide and preventing self aggregation of the peptide molecules leading to amyloid formation. In case of indole-3-carbinol, it is reported to inhibit Aβ fibrillogenesis at a much higher concentration compared to other inhibitor molecules, suggesting it may form some micellar structure which is necessary for amyloid inhibition [36]. Further, Ligand Scout analysis showed the key role of hydrogen bonding and hydrophobic interactions between the peptide and the inhibitor molecules. Table 2 shows the list of the eight inhibitor molecules with the free energies of docking with Aβ peptide arranged in decreasing order. It can be seen that curcumin exhibited the highest free energy of binding suggesting formation of a highly stable complex with Aβ peptide. This result also correlates with the reported invivo data showing curcumin as one of the most effective inhibitors of amyloid plaque in the Alzheimer's disease mice models [37].

Table 2: List of the selected inhibitor molecules with their free energies of docking with Aβ42.

Sl.No	Ligand Name	PubChem ID	Molecular Formula	Molecular Weight(gm)	2D Structure	Energy(kJ /mole)
1	Curcumin	CID 969516	$C_{21}H_{20}O_6$	368.3799		- 284.32
2	Benzofuran derivatives	CID 3259	$C_{20}H_8I_4O_5$	835.8924		-278.05
3	Resveratrol	CID 445154	$C_{14}H_{12}O_3$	228.24328		-207.12
4	2,4 Dinitrophenol	CID 1493	$C_6H_4N_2O_5$	184.10636		-171.36
5	Indole-3-carbinol	CID 3712	C_9H_9NO	147.17386		-157.31
6	Myo-Inositol	CID 892	$C_6H_{12}O_6$	180.15588		-147.79

7	4-hydroxyindole	CID 75421	C_8H_7NO	133.14728		-143.36
8	3-hydroxyindole	CID 50591	C_8H_7NO	133.14728		-139.98

To further gain insight into the mechanism of fibrillogenesis, docking study was done between two Aβ peptide molecules to identify the binding sites and type of intermolecular interactions. Figure 11 shows the docking result between two Aβ molecules. The results shows strong binding between the two peptide molecules with a binding energy of -640 kJ/mol. Also the amyloidogenic regions were found to be aligned close to each other, suggesting that the binding is mediated through the amyloidogenic stretches 16-21 and 32-36 of Aβ peptides. This observation further validates the amyloid inhibitory effect of the compounds by blocking the amyloidogenic regions which are involved in self assembly of the peptide.

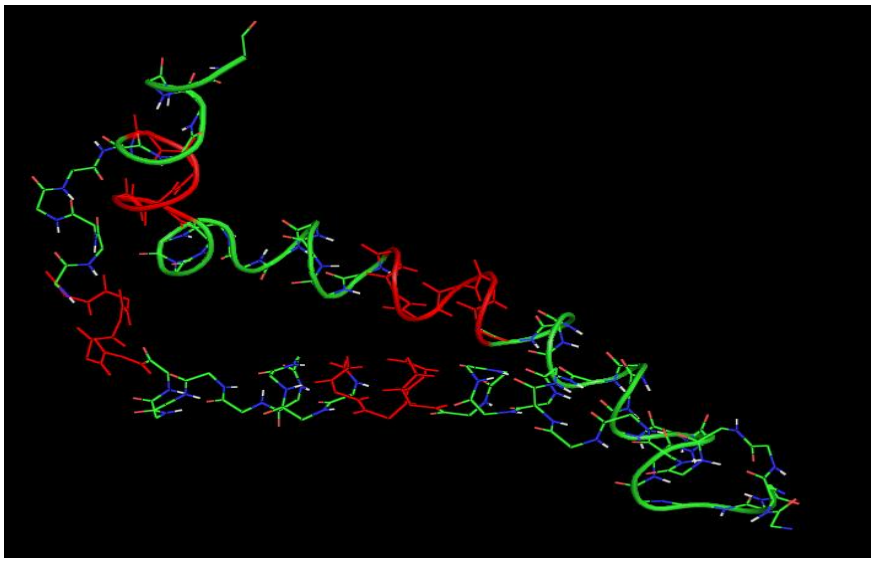


Figure 13: Docking complex between two Aβ (1-42) monomers.

CONCLUSION

The current study shows the potential binding sites of known amyloid inhibitor molecules on amyloid beta peptide, which is responsible for the onset of Alzheimer's disease. Eight inhibitor molecules were selected which showed significant inhibition towards Aβ amyloidogenesis *in vitro* but whose mechanism of action was unknown. The amino acid sequences in Aβ peptide, which are most prone to aggregation leading to amyloid formation, are predicted based on their hydrophobicities and secondary structure propensity, using a webserver named FoldAmyloid. Through molecular docking, most of these inhibitor molecules were found to bind within and in the vicinity of the predicted amyloidogenic regions of Aβ peptide thereby suggesting their mode of action by masking the amyloidogenic regions and preventing further self aggregation of the peptide. Further, Ligand Scout analysis showed the essential role of hydrogen bonding and hydrophobic interactions in stabilizing the peptide-inhibitor complex which might prevent further elongation of the fibrils.

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